Signal Without Solution? An Evidence-Based Review of Circulating Tumor DNA in Early-Stage Triple-Negative Breast Cancer

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Abstract

Background:

Circulating tumor DNA (ctDNA) is gaining interest as a potential biomarker for detection of molecular residual disease (MRD) and early identification of disease recurrence in patients with early-stage breast cancer- particularly, triple-negative breast cancer (TNBC). However, its role in guiding intervention to improve disease-free survival (DFS) remains unclear.

Objective:

To evaluate if ctDNA-guided intervention improves disease-free survival, compared to standard surveillance in patients with early-stage TNBC after completion of therapy with curative-intent.

Methods:

A structured, evidence-based review of selected clinical studies was conducted, with emphasis on the prognostic and potential clinical utility of ctDNA in early-stage, triple negative breast cancer. Analyzed studies included a prospective surveillance study (Garcia-Murillas et al.), a preplanned secondary analysis of a randomized clinical trial (Radovich et al.), and a phase II interventional trial evaluating ctDNA-guided therapy (Turner et al.). Primary outcomes included disease-free survival, overall survival, lead time to recurrence, and response to ctDNA-guided intervention.

Results:

Across studies, ctDNA detection is consistently associated with increased risk of recurrence and worse survival outcomes. In surveillance, ctDNA detection preceded clinical relapse by a median of approximately 10.7 months. In the Radovich et al. analysis, ctDNA positivity following neoadjuvant chemotherapy in patients with TNBC was associated with worse distant disease-free

survival (HR 2.99), disease-free survival (HR 2.67), and overall survival (HR 4.16), supporting its role as an independent prognostic marker. In the only prospective interventional study, a high proportion of patients had metastatic disease at the time of ctDNA detection, and ctDNA-guided treatment with pembrolizumab did not result in sustained ctDNA clearance or improved clinical outcomes.

Conclusions:

ctDNA is a strong prognostic marker in early-stage TNBC, capable of identifying patients at high risk of recurrence and demonstrating promise in surveillance. However, current evidence does not support its use to guide intervention to improve disease-free survival. Limitations in timing of detection, short lead time to relapse, and lack of effective intervention strategies may restrict its clinical utility. Further research is needed to optimize surveillance strategies and evaluate earlier or more effective ctDNA-guided interventions.

Introduction

Triple-negative breast cancer (TNBC) is a disease with negative immunochemistry for human epidermal growth factor receptor 2 (HER2 0-1+), estrogen receptor (ER <1%), and progesterone receptor (PR <1%). TNBC comprises roughly 15-25% of all breast cancers and is notorious for its distinct molecular profile, aggressive nature, and current lack of targeted treatment options. This type of breast cancer has a strong correlation with BRCA 1 and BRCA 2 mutations, making genetic history an identifiable risk factor. African American and Hispanic women tend to have a higher risk of disease incidence; compared to other ethnic groups, African

American women are more likely to experience worse clinical outcomes. Early-stage cancer includes stages I-III, with stage IV representing metastatic disease. TNBC commonly follows a metastatic pattern and naturally favors recurrence, ultimately leading to poor prognoses. Patients typically demonstrate a 65% 5-year survival rate with regional disease and 11% in those with metastases to distant organs such as the brain, lungs, or bones. Currently, there is no cure for metastatic TNBC and metastasis remains one of the strongest indicators of mortality among affected patients.

Primary modalities of treatment include conventional chemotherapy, surgery, and radiation. Though minimal approved options for targeted therapy exist, combining various types has shown promise and is continuing to be explored in clinical studies. Following completion of therapy with curative intent, patients will begin to undergo a surveillance regimen in order to detect recurrence and implement necessary, timely intervention. At this time, surveillance is monitored with clinical follow-up and imaging. These methods often detect recurrence only as it becomes clinically evident, which may limit the possibility of earlier intervention.

Measuring molecular residual disease (MRD) in cancer patients, both active and in remission, serves as a pivotal aspect in therapy optimization and assessment of relapse risk. Current methods of MRD detection include next generation sequencing, flow cytometry, and polymerase chain reaction, but each of these possess unique limitations in achieving both optimal sensitivity and specificity. In patients with solid tumors such as breast cancer, circulating tumor DNA (ctDNA) was recently introduced and explored as a biomarker for disease prognostication and monitoring at a molecular level. ctDNA includes fragments of solid tumor DNA that enter

the bloodstream and can be detected via liquid biopsy of peripheral blood and molecular assays. At this time, the utilization of ctDNA in identifying minimal residual disease before it can be identified on imaging or physical exam suggests significant potential in malignancy prognostication. However, little is currently known about its utility in guiding therapeutic intervention during periods of disease surveillance.

The question this evidence-based review seeks to explore is: For patients with early stage triple-negative breast cancer who have completed neoadjuvant or curative-intended therapy, does circulating tumor DNA (ctDNA)-guided intervention, compared with standard surveillance practices, improve disease-free survival?

Methods

A systematic literature search was conducted to identify clinical studies that investigate the use of ctDNA for patients with early stage triple-negative breast cancer. Online medical databases such as PubMed, Cochrane Library, and Embase were utilized using search criteria keywords such as, “ctDNA,” “circulating tumor DNA,” “minimal residual disease,” “early stage triple-negative breast cancer,” “surveillance,” and “recurrence.” AND/OR functions were applied to synthesize and refine the results. The PubMed Clinical Queries tool was utilized to apply these keywords in both broad and narrow scopes. Studies were refined to peer-reviewed journals in English with human participants over the age of 18, resulting in 36 initial studies.

Inclusion criteria was established within more specific parameters as it relates to ctDNA and surveillance of early stage TNBC following therapy with intent to cure. Patients had to be older than 18 years of age and diagnosed with stage I-III TNBC as defined by immunochemistry. Curative-intended therapy, neoadjuvant or adjuvant, must have been completed in all study participants. Patients must have been evaluated within a surveillance-focused time frame following therapy completion. During the surveillance period, ctDNA biomarker assays must have occurred to evaluate minimal residual disease and/or prompted clinical intervention. Study outcomes were required to report clinically meaningful assessments of relapse, recurrence, and/or disease-free survival with respect to ctDNA monitoring.

Selection criteria for this analysis excluded case reports, expert opinion papers, conference abstracts without full published data, and editorials. Clinical studies involving patients with metastatic disease or lacking discussion of results with recurrence implications were excluded. Studies involving non-TNBC breast cancer pathologies or exploring ctDNA only as a diagnostic marker prior to treatment completion were also excluded. Clinical outcome data related to ctDNA during a post-treatment surveillance period was required.

The initial database search yielded 36 studies, reviews, and analyses. Following screening and application of inclusion and exclusion criteria, nine studies met requirements. Ultimately, three studies were selected for further evaluation based on their direct relevance to the research question. These studies include Garcia-Murillas et al. (2019), N.C. Turner et al. (2023), and Radovich et al. (2020); a prospective cohort study, prospective interventional trial, and secondary analysis of randomized clinical trial (RCT), respectively.

A literature summary report (see table 1) was created to evaluate study design, patient population, detection methods, sampling timeline, related clinical outcomes, and strength of evidence as it relates to ctDNA and disease recurrence. Additionally, an assessment of study validity and quality (see table 2) was performed in adherence to criteria such as meaningful endpoint, blinding, patient follow-up, intent to treat, comparison groups, safety outcomes, and degree of statistical power. Each aspect was graded by its adherence to the aforementioned criteria by adequate (A), marginal (M), or inadequate (I). Study quality was further organized into high (H), moderate (M), low (LQ), or inadequate (IQ).

Results

Across the included studies, ctDNA detection following completion of neoadjuvant or curative-intended therapy is consistently associated with an increased risk of disease recurrence. However, despite the demonstration of its prognostic value, there is currently no evidence that ctDNA detection can be used to guide interventions that improve overall or disease-free survival in patients with early stage triple-negative breast cancer.

Molecular Relapse Detection with ctDNA in Early Stage Breast Cancer, Garcia et. al:

Design:

This is a prospective, multi sample collection validation study conducted among 5 medical centers in the United Kingdom. The purpose of this study is to assess the validity of molecular relapse with the utilization of ctDNA as a clinically meaningful biomarker in patients with early stage breast cancer.

Conduct:

The main cohort of this study includes 101 patients that have undergone either adjuvant or neo-adjuvant therapy with curative intent. The primary cohort was derived from the initial enrollment of 170 individuals based on the identification of trackable tumor-specific genetic mutations. Beyond this, a secondary cohort of 144 is established with the inclusion of 43 additional patients from a previously published study. Each patient's primary tumor is sequenced to identify somatic mutations that comprise its ctDNA and a unique polymerase chain reaction assay is developed for monitoring potential relapse throughout the trial. During the first year, plasma samples are taken and analyzed using the digital assay every 3 months. For up to 5 years beyond this time frame, samples are being collected and tested every 6 months. Of note, buffy coat DNA is analyzed to control for clonal hematopoiesis, which can lead to false positives.

Results:

Throughout the follow-up period, detection of ctDNA is closely associated with recurrence of disease. Among the 170 patients initially enrolled, somatic mutations suitable for tracking are identified in 101 patients, who form the primary analysis cohort. Across this cohort, 695 plasma samples are collected and analyzed longitudinally, with a median of 7 samples per patient.

In the primary cohort, ctDNA detection during follow up is associated with significantly worse relapse-free survival (HR 25.2; 95% CI, 6.7–95.6; $p < 0.001$). During follow up, molecular residual disease (MRD) is detected in 16 of 101 patients (15.8%), and these patients experience substantially worse outcomes compared to those without detectable ctDNA. Median relapse-free survival is 38.0 months in patients with ctDNA-detected MRD. The median is not reached in ctDNA-negative patients, indicating that the majority of ctDNA-negative patients remain free of recurrence during the study time frame.

At baseline, prior to initiation of therapy, ctDNA is detected in 41 of 80 evaluable patients (51.2%) which reflects the subset of patients with available plasma samples. Detection of ctDNA at diagnosis is associated with increased risk of relapse (HR 5.8; 95% CI, 1.2–27.1; $p = 0.01$).

In the combined cohort of 144 patients, ctDNA detection precedes clinical relapse by a median of 10.7 months (95% CI, 8.1–19.1). ctDNA identifies recurrence in 23 of 29 patients (79%) overall, including 22 of 23 patients (96%) with extracranial metastatic disease, demonstrating high sensitivity for systemic relapse. Detection is reduced in patients with metastases exclusive to the brain, with ctDNA identifying 1 of 6 cases (17%), suggesting a limitation in detecting certain sites of disease recurrence.

Disease subtype analysis demonstrates that ctDNA detection remains highly prognostic across all major breast cancer subtypes. In patients with triple-negative breast cancer, ctDNA positivity is associated with significantly worse relapse-free survival (HR 27.6; 95% CI, 5.9–128.8; $p < 0.001$), with a median lead time to relapse of approximately 10.6 months. Additionally, ctDNA levels at diagnosis are the highest in patients with triple-negative disease, with a median of 4.96 copies/mL, compared with lower levels observed in other subtypes.

Overall, these findings demonstrate that ctDNA detection identifies a subset of patients at significantly increased risk of recurrence and provides a measurable lead time prior to clinically detectable disease.

Conclusions:

This study demonstrates that the surveillance and subsequent detection of ctDNA is meaningfully associated with increased relapse risk. Further, the identification of molecular residual disease can occur months prior to clinical recurrence. Overall, the findings from this study support the validity of ctDNA as a clinically meaningful biomarker in prognosticating patients with early stage breast cancer, as well as its potential use in surveillance strategies moving forward.

Limitations:

This study is conducted across multiple academic institutions without evidence of direct industry sponsorship. However, specialized laboratory techniques were required which may introduce subtle sources of bias. While the overall risk of sponsorship bias appears low, these factors should be taken into consideration. The observational design of this study does not allow for a control group and randomization, therefore lacking exploration of ctDNA-guided intervention as it relates to the improvement of clinical outcomes. Although the sample size is moderate, the study population is heterogeneous in nature and includes multiple breast cancer subtypes rather than a specific focus on triple-negative breast cancer, which may limit applicability of findings to this high-risk population. Discrepancies are also noted in the detection rates at different metastatic sites, particularly the brain. The limitations of this study suggest that the utility of ctDNA in guiding treatment decisions amidst surveillance remains unproven.

Post-Neoadjuvant Chemotherapy ctDNA in Triple-Negative Breast Cancer, Radovich et. al:

Design:

This study is a preplanned secondary analysis from the BRE12-158 trial, a phase 2 multicenter randomized clinical trial involving patients with early stage triple-negative breast cancer and molecular residual disease following neoadjuvant chemotherapy. In the parent trial, patients are randomized to receive post-neoadjuvant genomically directed therapy or physician's choice standard-of-care treatment. The objective of this study is to explore if ctDNA and circulating tumor cells (CTC) detected after neoadjuvant chemo is associated with recurrence and survival outcomes in patients with residual disease.

Conduct:

This paper utilizes data from the BRE12-158 parent trial, which includes 196 patients with early stage TNBC and identified residual disease after the completion of neoadjuvant chemotherapy. At the time of patient randomization, blood samples are collected from participants and ctDNA sequencing is performed using FoundationACT or FoundationOne liquid assays. Circulating tumor cells are measured using an epithelial cell adhesion molecule-based, positive selection microfluidic device. Of the 196 patients included in the parent study, ctDNA analysis with survival data is found in 142 patients; CTC analysis with survival data is identified in 123 patients. After the initial ctDNA analysis, patients are routinely followed using standard clinical surveillance methods rather than specific points in time. The median follow-up timeframe among patients is 17.2 months. Despite this timeframe, Kaplan-Meier statistical analyses are applied to

estimate survival probability at 24 months. Studied outcomes include distant disease-free survival (DDFS), disease-free survival (DFS), and overall survival (OS). Recurrence is assessed in participants throughout the study as indicated by routine clinical follow-up and imaging or the development of new symptoms.

Results:

Identifiable post-neoadjuvant treatment ctDNA is strongly associated with poorer clinical outcomes overall. Among the 196 patients enrolled in this study, ctDNA analysis with survival data is available for 142 patients with a median follow-up of 17.2 months. Patients with initially detected ctDNA are found to have worse distant disease-free survival (DDFS), with a median DDFS of 32.5 months in ctDNA positive patients, compared to not reached in ctDNA-negative patients (HR 2.99; 95% CI, 1.38-6.48; *P* =.006). At 24 months, the probability of DDFS is 56% in ctDNA-positive patients compared with 81% in ctDNA-negative patients, demonstrating a substantial absolute difference in outcomes.

Similarly, ctDNA positivity is also associated with inferior disease-free survival (DFS), with a median DFS of 22.8 months in ctDNA-positive patients compared to not reached in ctDNA-negative patients (HR 2.67; 95% CI, 1.28-5.57; *P* = .009). At 24 months, DFS probability is 50% in ctDNA-positive patients compared to 76% in ctDNA-negative patients.

Overall survival (OS) is reduced in ctDNA-positive patients (HR 4.16; 95% CI, 1.66-10.42; *P* = .002). At 24 months, the OS probability is 57% in ctDNA-positive patients and 80% in ctDNA-negative patients.

The combination of both ctDNA and CTCs provides additional prognostic information with survival data for 123 patients. While CTC positivity alone is not significantly associated with outcomes, increasing CTC burden is associated with worse survival; patients who are positive for both ctDNA and CTCs demonstrate the poorest outcomes. Those who are both ctDNA and CTC positive demonstrate worse DDFS than patients negative for both biomarkers (HR 5.29; 95% CI, 1.50-18.62; P = .009), with 24-month DDFS probabilities of 52% and 89%, respectively. Additionally, similar trends are identified for DFS (HR 3.15; 95% CI, 1.07-9.27; P = .04) and OS (HR 8.60; 95% CI, 1.78-41.47; P =.007).

It is important to recognize that ctDNA and CTC positivity are not strongly correlated (p = 0.19), suggesting that these biomarkers may provide complementary information. Combining ctDNA and CTC detection improves sensitivity for identifying recurrence. ctDNA alone identifies recurrence in 79% of patients (23 of 29), while CTCs alone identify 62% (18 of 29). When used together, detection increases to 90% (26 of 29 patients), demonstrating improved ability to identify patients at highest risk of relapse.

Conclusions:

The results of this study suggest that, in patients with early stage triple-negative breast cancer, ctDNA detection after completion of neoadjuvant chemotherapy is independently associated with recurrence of disease and poorer survival outcomes. These findings support the idea that ctDNA may be a useful component in risk stratification for future post-neoadjuvant studies.

Limitations:

This study is conducted as a secondary analysis of a multi-center randomized clinical trial and utilizes commercially available ctDNA sequencing platforms from Foundation Medicine Incorporated. The study itself is predominately academic, though the use of industry diagnostic assays may introduce potential bias related to performance, sensitivity, and interpretation of sequencing results. This should be considered when evaluating the reported significance of ctDNA prognostication. This study does not evaluate ctDNA in the context of longitudinal surveillance, but instead assesses ctDNA at a single point in time after the completion of neoadjuvant chemotherapy. This study is a secondary analysis, meaning that it is not designed to explore if ctDNA-guided intervention improves outcomes. Rather, it explores the relationship between initial presence of ctDNA and patient outcomes over time. Follow-up was relatively short, at a median of 17.2 months, which may limit the identification of long term recurrence patterns. In addition, the impact of different post-neoadjuvant therapies and surveillance is not fully controlled for in this analysis. While this study provides meaningful prognostic evidence, it does not fully reflect the clinical utility of ctDNA or support its routine use to guide clinical decision making.

c-TRAK TN Clinical Trial, Turner et, al

Design:

The c-TRAK TN study is a multi-center, phase II clinical trial with both prospective ctDNA surveillance as well as therapeutic intervention components. This is the first prospective study to assess if ctDNA assays are able to identify molecular residual disease in patients with early stage triple-negative breast cancer as it relates to the clinical utility of ctDNA-guided interventions.

There were two main participant groups included in this study; early stage TNBC with MRD following neoadjuvant chemotherapy and high risk disease following surgery with adjuvant chemotherapy. Groups were established for moderate risk and high risk based on both primary tumor and nodal characteristics.

Conduct:

208 total participants are initially included in this study, with 185 having tumor tissue sequencing performed. From there, 171 patients have identifiable mutations and 161 patients enter prospective ctDNA surveillance. Personalized digital PCR ctDNA assays are developed to track patients' tumor-specific mutations. Every 3 months, blood samples are collected and analyzed for ctDNA tracking. This process continues for up to 24 months, though most active surveillance occurs within the first year. The median follow-up for this study was 20.4 months.

The detection of ctDNA prompts randomization in a 2:1 ratio to intervention or observation. Patients in the intervention group are undergoing imaging to stage their disease. Those without evidence of metastasis are offered pembrolizumab 200 mg every 3 weeks, for up to 1 year with ctDNA collection at each cycle. This reflects a standard dosing regimen for Pembrolizumab, though its use in a ctDNA-guided post-treatment setting in early-stage TNBC is not yet established in clinical practice. Patients with metastatic disease confirmed by imaging are treated off trial with standard-of-care protocol. Patients in the observation group continue with the study's protocol of ctDNA surveillance and routine follow-up. Of note, a later protocol amendment in September 2020 closed the observation group of this study. As a result, all ctDNA-positive patients have been assigned to the intervention group.

Primary outcomes of this study include risk prediction with rates of ctDNA detection, as well as sustained ctDNA clearance following pembrolizumab treatment. Secondary outcomes include time to ctDNA detection, time to relapse, and relevant safety of pembrolizumab therapy.

Results

Within the first 12 months of surveillance, ctDNA is detected in 27.3% of patients (44/161; 95% CI, 20.6%-34.9%). Higher detection rates are observed in high-risk patients, with Kaplan-Meier statistical analyses estimating 55.7% in high-risk patients versus 11.8% in moderate-risk patients at 12 months. At baseline, 37.0% (20/54) of high-risk patients are ctDNA positive compared with only 2.8% (3/107) of moderate-risk patients.

Among patients who became ctDNA-positive, 45 subsequently entered the therapeutic component of this trial. Of note, 71.9% (23/32; 95% CI, 53.3%-86.3%) of patients assigned to the intervention group were found to have metastatic disease on staging imaging at the time of ctDNA detection.

Of all patients without identified metastatic disease, very few moved forward with treatment; only 5 patients initiated Pembrolizumab and none achieved sustained ctDNA clearance at 6 months. All of these patients experienced recurrence of disease, with a median time from ctDNA detection to recurrence of 1.6 months (95% CI, 1.2-4.9 months) in the intervention group. Adverse effects related to pembrolizumab are reported as consistent with prior patient experience and generally manageable, including fatigue, rash, and immune-related adverse events such as thyroid dysfunction. The sample of 5 treated patients is too small to allow for statistically significant conclusions.

In the observation group, the median time from ctDNA detection to recurrence is 4.1 months (95% CI, 3.2 months-not defined), with 21.4% (3/14) of patients demonstrating ctDNA clearance at 6 months. The study suggests that this finding likely reflects detection variabilities rather than true eradication of disease.

7 patients without prior detection of ctDNA experienced disease recurrence, indicating that surveillance of ctDNA did not fully capture all clinical relapse and suggesting limitations in sensitivity.

Conclusions

This study demonstrates that prospective ctDNA surveillance is feasible in early-stage TNBC and can identify patients with molecular residual disease and a high risk of recurrence. However, a high proportion of patients are found to have metastatic disease at the time of ctDNA detection. In the small number of treated patients, it has been found that ctDNA-guided intervention with pembrolizumab does not result in sustained ctDNA clearance or improved outcomes. These findings suggest that while ctDNA has prognostic value, its role in guiding early clinical intervention remains uncertain and may be limited by the timing of ctDNA detection. However, this study also emphasizes that future trials should initiate surveillance earlier and possibly with a more sensitive testing approach.

Limitations

Although this trial is sponsored by an academic research institution, The Institute of Cancer Research, it involves the use of Pembrolizumab, which is an immunotherapy agent developed by Merck Biopharmaceutical Company. While there is no direct evidence of manufacturer involvement or bias, the utilization of a targeted therapeutic drug and specific assay techniques

may introduce other potential sources of indirect bias. Further, the small fraction of patients receiving the intervention limits the ability to draw significant conclusions regarding the treatment's efficacy. This study is limited by the unexpectedly significant number of patients with metastatic disease identified at the time of first ctDNA detection, which reduces the number of patients eligible for pembrolizumab intervention. Regardless, only 5 patients ultimately pursue therapy with pembrolizumab, which prevents meaningful evaluation of this treatment. This study is not adequately powered to compare intervention and survival outcomes. Modifications to the observation group during this trial may introduce bias. It is possible that a 3 month testing sequence is not sensitive or frequent enough to detect disease in early stage TNBC. Although this study demonstrates the feasibility of ctDNA-guided surveillance and evaluates its use in intervention, it does not demonstrate meaningful outcomes or establish clinical advantages from ctDNA-guided treatment.

Discussion

The findings of this evidence-based review assess the role of circulating tumor DNA in guiding surveillance in patients with early stage triple-negative breast cancer following therapy with curative intent. It is demonstrated across these studies that the detection of ctDNA is consistently associated with an increased risk of recurrence and worse survival outcomes, though the ability to identify molecular relapse prior to clinical detection is promising. ctDNA continues to function as a strong prognostic marker, identifying patients with molecular residual disease (MRD) who are at a significantly higher risk of recurrence following the completion of therapy.

Despite the promising nature of these findings, current evidence is not sufficient to support the routine use of ctDNA-guided intervention to improve disease-free survival.

The study by Garcia-Murillas et al. supports the role of ctDNA in a surveillance context, demonstrating a significant lead time between ctDNA detection and clinical relapse with a median lead time of about 10 months. These findings suggest that ctDNA may allow for earlier identification of recurrence compared to standard clinical follow-up. Further, the analysis by Radovich et al. reinforces the prognostic significance of ctDNA in a high risk TNBC patient population, demonstrating that ctDNA positivity after neoadjuvant chemotherapy is independently associated with worse distant disease-free survival, disease-free survival, and overall survival. These studies synergize to provide strong evidence that ctDNA is a reliable marker of recurrence risk.

While ctDNA demonstrates clear prognostic value, its role in guiding intervention remains unclear. The c-TRAK TN trial by Turner et al. represents the only included study to prospectively evaluate both ctDNA-guided surveillance and intervention. Although ctDNA surveillance successfully identifies patients at high risk of relapse, a large proportion of patients are found to have metastatic disease at the time of detection. Among patients who are eligible for treatment, pembrolizumab does not result in sustained clearance of ctDNA or improvement in clinical outcomes. These findings suggest that ctDNA detection may occur too late in disease relapse to allow for meaningful intervention.

The time frame surrounding ctDNA detection is shown to be an important factor influencing clinical utility. The Turner trial demonstrates a much shorter interval between ctDNA detection and clinical relapse than prior studies, particularly in high risk TNBC populations. These findings may reflect the aggressive nature of TNBC or any potential delays in the initiation of ctDNA surveillance following therapy completion. As a result, it is suspected that ctDNA detection may precede clinically detectable metastatic disease more closely, further limiting the window for therapeutic intervention.

Variability in ctDNA detection sensitivity must be considered. Across studies, a subset of patients experience recurrence without prior ctDNA detection; this suggests that current assays may not detect all cases of MRD. Instances of ctDNA clearance without treatment raises concerns regarding assay variability and the potential for false-positives. These factors further complicate interpretation of ctDNA results in clinical practice. Several additional limitations were identified through the validity assessment. The majority of studies were observational in design and lacked randomization and blinding, increasing the risk of bias. Variability in ctDNA assay methodologies, detection thresholds, and timing of sample collection limits comparison across studies. Small sample sizes in these studies reduce statistical power and limit the ability to detect meaningful differences in clinical outcomes.

The current evidence supports ctDNA as a valuable prognostic and potential surveillance tool that can identify patients at high risk of recurrence, but it does not establish clinical utility in improving disease-free survival through ctDNA-guided interventions compared with current standard-of-care surveillance.

Future research should focus on optimizing the timing and frequency of ctDNA surveillance, improving assay sensitivity, and evaluating earlier or more effective intervention strategies; all of which should include adequately powered, randomized trials. Initiating ctDNA monitoring earlier in the treatment course and incorporating more sensitive detection methods may improve the ability to identify MRD prior to the development of apparent metastatic disease. Ongoing and future clinical trials will be pivotal in exploring if ctDNA-guided approaches can ultimately improve clinical outcomes in patients with early-stage TNBC.

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Table 2: Summary of Study Characteristics and Outcomes

Study / Design	Total Patients	ctDNA Assessment	Comparison	Patient Timeline	Efficacy Outcome	Safety Outcome
Garcia-Murillas et al., 2019 (Prospective Cohort)	~101	Serial post-treatment ctDNA monitoring	ctDNA positive vs negative	Median follow-up ~2–3 years	HR for relapse **25.2** ($p<0.001$); ctDNA detected relapse **10.7 months earlier**	No primary outcome; no significant safety concerns reported

Radovich et al., 2020 (Secondary Analysis of RCT)	~196	ctDNA after neoadjuvant chemotherapy	ctDNA positive vs negative	Median follow-up 17.2 months	DDFS: **56% vs 81%** (ctDNA+ vs ctDNA−)	No primary outcome; no major safety issues reported
Turner et al., 2023 (c-TRAK TN, Prospective Interventional Trial)	~161 screened; fewer ctDNA+ treated	ctDNA-guided intervention (pembrolizumab)	Standard surveillance/ no ctDNA-guided intervention	Variable; ~12–24 months	Feasibility demonstrated; **no clear DFS benefit**	Immunotherapy-related adverse events reported; manageable

Table 4: Study Validity Assessment

Study	Study Design	Meaningful Endpoint	Blinding	Meaningful Comparison	Adequate Timeline	Patient Accounting	Intention to Treat	Power	Safety	Grade
Garcia-Murillas et al., 2019	Prospective Cohort	A	I	M	A	A	I	M	M	MQ
Radovich et al., 2020	Secondary RCT Analysis	A	M	A	A	A	A	M	M	MQ
Turner et al., 2023 (c-TRAK TN)	Prospective Interventional Trial	A	M	A	M	M	M	I	A	MQ

Figure 1: ctDNA Detection Timeline in Early Stage TNBC
ctDNA detection of recurrence may precede clinical recurrence by 3-12 months

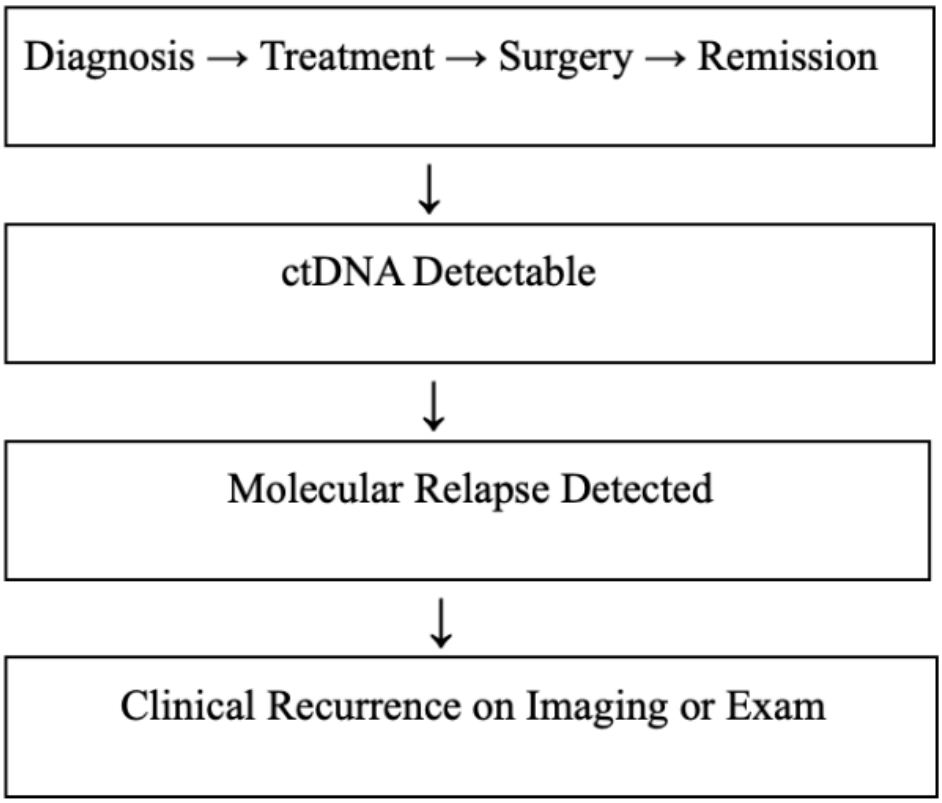

Figure 2: ctDNA Detection Timeline in Surveillance of Early Stage TNBC

Clinical Stage	ctDNA Status	Clinical Findings	Significance
Initial Diagnosis	Detectable	Tumor identified by biopsy/imaging	ctDNA reflects tumor burden
Neoadjuvant Chemotherapy	Declining ctDNA levels	Tumor response to treatment	ctDNA clearance may predict treatment response
Post-Surgery / Curative Therapy	Undetectable ctDNA	No clinical evidence of disease	Indicates possible complete response
Minimal Residual Disease Phase	ctDNA becomes detectable again	Imaging still negative	Indicates **molecular residual disease**
Molecular Relapse	Increasing ctDNA levels	Patient asymptomatic	ctDNA detects recurrence **months prior to clinical exam or imaging**

Clinical Recurrence	High ctDNA levels	Imaging confirms metastasis	Standard surveillance detects disease